REGULATION OF PROSTAGLANDIN SECRETION FROM EPITHELIAL AND STROMAL CELLS OF THE BOVINE ENDOMETRIUM BY INTERLEUKIN-1β, INTERLEUKIN-2, GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR AND TUMOR NECROSIS FACTOR-α

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Summary

Bovine endometrium was obtained on day 16 of pregnancy (estrus = 0) and separated into epithelial and stromal cell populations. When confluent, the two cell populations were treated for 24 h with cytokines at 1, 10 and 100 ng/ml. Prostaglandin (PG) E₂ was the major prostaglandin produced by both cell types. For control cultures, more PGE₂ was secreted into medium by stromal cells than by epithelial cells, whereas secretion of PGF was similar for epithelial and stromal cells. Interleukin-1β had no effect on prostaglandin production by stromal cell cultures but increased epithelial production of PGE₂ and, to a lesser extent, PGF. Conversely, granulocyte-macrophage colony stimulating factor had no effect on epithelial cells but reduced secretion of PGE₂ and PGF from stromal cells. There were no effects of interleukin-2 or tumor necrosis factor-α on prostaglandin secretion. Results indicate that certain cytokines can regulate endometrial prostaglandin secretion in a cell type-restricted manner.

Many regulatory polypeptides originally identified as being of immune origin are also produced in tissues of the uterus and placenta. Among the molecules reported to be produced by uterine or placental tissues are interleukin-1 (IL-1) (1-3), granulocyte-macrophage colony stimulating factor (GM-CSF) (4), tumor necrosis factor (5,6), transforming growth factor-β (7), colony stimulating factor-1 (7), IL-6 (8) and interferon-α (9). In light of the possibility that a proximal source of cytokines exists within the uterus, it is possible that several of these could regulate the in vitro secretion of prostaglandins by epithelial and stromal cells of bovine endometrium. Interleukin-1 has been shown to be a potent stimulator of PGE₂ secretion from several tissues including human uterine epithelial cells (10) and decidua (11). Tumor necrosis factor also can increase PGE₂ synthesis by decidua (11). While not tested on uterine or placental tissues, both IL-2 (12) and GM-CSF (13,14) can increase PGE₂ synthesis in other tissues. Prostaglandins play a key role in several aspects of pregnancy including regulation of luteal function (15-17), blastocyst hatching (18), blood flow (19), endometrial vascular permeability (20), immunoregulation (21), and parturition (22). Regulation of their secretion could represent one mechanism by which locally-produced cytokines regulate uterine-conceptus function. In the present experiment, the effects of these cytokines on prostaglandin synthesis by endometrium from cows at day 16 of pregnancy was tested. Day 16 represents a critical period of pregnancy in the cow: the embryo acts upon the endometrium to block PGF₂α secretion at this time and thereby prevents luteolysis (15). By the end of the third week of gestation, maternal-conceptus attachment has been initiated (23).

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Methods

Materials. Ultra-Ser SF serum substitute was obtained from IBF Biotechnics (Columbia, MD). Iron-supplemented calf serum and horse serum were obtained from Hy-Clone (Logan, UT), collagenase from Worthington (Freehold, NJ) and Falcon tissue culture plates from Fisher (Pittsburgh, PA). Recombinant bovine IL-1β (IL-1) was a generous gift from American Cyanamid (Princeton, NJ). Other recombinant bovine cytokines [IL-2, GM-CSF and tumor necrosis factor-α (TNF-α)] were generously donated by CIBA-GEIGY, Basle, Switzerland. Spectra/Mesh nylon filters were obtained from Spectrum Medical Industries, Inc. (Los Angeles, CA). Culture medium, antibodies to cytokeratin (clone KB-37) and vimentin (clone V9) and other reagents were obtained from Sigma (St. Louis, MO).

Cell isolation. Cells were isolated from the endometrium of pregnant Holstein cows on day 16 of pregnancy (estrus = day 0). Intact reproductive tracts were obtained from cows immediately following exsanguination. Further processing of tracts for tissue culture was performed in sterile conditions under a laminar flow hood. Uterine endometrium was dissected from the body and both horns of the uterus, minced very finely and dispersed in 300 ml of Earle’s Balanced Salts containing 150 U/ml Type IV collagenase, 150 U/ml hyaluronidase and 2% (w/v) bovine serum albumin (BSA) followed by incubation at 37°C in 5% CO₂ for 2 hr with gentle agitation. After washing and trituration 3 times to remove residual enzymes and promote further dissociation of tissue, cells were filtered through 4 layers of cheesecloth to remove connective tissue and large clumps of cells. The resulting filtrate was then passed through a nylon filter (30 μm mesh). Retained cells were predominately epithelial in nature while the filtrate consisted primarily of stromal cells. For one preparation of cells, presence of epithelial and stromal cells and purity of cell preparations was confirmed by staining wells of epithelial cells and stromal cells with antibodies to cytokeratin and vimentin using procedures described elsewhere (24).

Cell culture. Epithelial cells and stromal cells were plated in 6-well plastic tissue culture plates at an optical density of approximately 0.2-0.3 (epithelial cells) or 0.05-0.1 (stromal cells) in a formulation consisting of 40% Eagle’s minimum essential medium (MEM), 40% Ham’s F-12, 10% fetal calf serum and 10% heat-inactivated horse serum (v/v). Plating medium was further supplemented with 0.1 IU/ml insulin and 1% (v/v) antibiotic-antimycotic (ABAM) solution (10,000 U/ml penicillin G, 10 mg/ml streptomycin and 25 μg/ml amphotericin B). Epithelial and stromal cells were allowed to attach for 36 and 24 hr, respectively, at which time medium was changed to 49% MEM, 49% Ham’s F-12 and 2% Ultra-Ser SF (v/v) supplemented with insulin and ABAM.

Treatments. When cells became confluent (at 5-7 days of culture), medium was changed to 50% MEM and 50% F-12 supplemented with ABAM. Insulin and Ultra-Ser SF were omitted. Cytokines were added at final concentrations of 0, 1, 10 or 100 ng/ml and cells cultured for 24 hr. At the end of the culture period, medium was harvested and frozen at -20°C until prostaglandin radioimmunoassays (RIA) were performed. Treatments were applied to quadruplicate wells for each culture. Additionally, each experiment was replicated using cells from 2 (IL-1, stroma), 3 (IL-1, epithelium and GM-CSF, stroma) or 4 (other treatments) separate cows.

Analytical procedures. Medium was assayed for PGE₂ and PGF₂α using previously described methods (25). Since the antibody to PGF₂α cross-reacts with PGF₁α, data are expressed as PGF.

Statistical analysis. Data were analyzed by least-squares analysis of variance using the General Linear Models procedure of the Statistical Analysis System (SAS Inst., Cary, NC). Effects of each cytokine were determined for each cell type separately using effects of cow, cytokine concentration and cow x concentration interaction. Orthogonal contrasts were used to partition effects of
concentration into linear, quadratic and cubic components. Also, Duncan's multiple range test was performed to determine which concentrations differed from each other.

**Results**

**Characteristics of epithelial and stromal cells.** To determine characteristics of prostaglandin secretion for epithelial and stromal cells, prostaglandin concentrations were assessed for wells not receiving any cytokines. For both tissues, PGE$_2$ was the predominant prostaglandin produced. There were, however, differences between cell types in the amounts of PGE$_2$ and PGF produced. Concentrations of PGE$_2$ after 24 h of culture (means ± between-cow SEM) were less (P<0.001) for epithelial cells (40.0 ± 13.9 ng/ml) than for stromal cells (107.9 ± 40.0 ng/ml). In contrast, epithelial cells produced PGF in similar quantities as stromal cells (14.4 ± 10.7 pg/ml and 10.7 ± 8.2 ng/ml, respectively).

**Effects of IL-1.** Secretion of PGE$_2$ (P<0.005) and PGF (P<0.08) from epithelial cells was increased by IL-1 (Fig. 1). The increase was greater for PGE$_2$; concentrations of PGE$_2$ increased from 33.4 ± 11.5 ng/ml without IL-1 to 90.3 ± 12.2, 105.4 ± 11.5 and 104.2 ± 11.5 ng/ml with 1, 10 and 100 ng/ml IL-1, respectively (least squares means ± SEM). All concentrations of IL-1 were equally effective in increasing PGE$_2$. For PGF, in contrast, concentrations were 18.5 ± 2.2 ng/ml (least squares mean ± SEM) for cultures without IL-1 and 21.6 ± 2.4, 26.3 ± 2.3 and 24.2 ± 2.3 ng/ml for cultures with 1, 10 and 100 ng/ml IL-1, respectively. Only the 10 ng/ml IL-1 treatment differed (P<0.10) from control cultures. For both prostaglandins, the increase in secretion caused by IL-1 was observed for all cultures.

In contrast to its effect on epithelial cells, there was no effect of IL-1 on secretion of PGE$_2$ or PGF by stromal cells (Fig. 1). Concentrations of PGE$_2$ averaged 62.1 ng/ml for cells without IL-1 and 44.7-59.8 ng/ml for cells with IL-1. Concentrations of PGF averaged 2.3 ng/ml for cells without IL-1 and 1.8-2.4 ng/ml for cells with IL-1.

**Effects of GM-CSF.** GM-CSF had no effect on secretion of PGE$_2$ or PGF from epithelial cells but caused a decrease in secretion of PGE$_2$ (P<0.02) and PGF (P<0.05) from stromal cells. For PGE$_2$, least squares means (± SEM) were 72.8 ± 7.1, 47.3 ± 7.5, 44.3 ± 7.9 and 37.6 ± 7.9 ng/ml for 0, 1, 10 and 100 ng/ml GM-CSF, respectively. Mean concentrations of PGE$_2$ were similar for wells treated with 1, 10 and 100 ng/ml GM-CSF and these values were different than values for control wells without GM-CSF. For PGF, least squares means (± SEM) were 2.5 ± 0.33, 2.0 ± 0.38, 1.6 ± 0.33 and 1.5 ± 0.33 ng/ml for 0, 1, 10 and 100 ng/ml GM-CSF, respectively. Only the value for 100 ng/ml differed from the value for cells without GM-CSF. For both PGE$_2$ (P<0.08) and PGF (P<0.02), there was a cow x concentration interaction because GM-CSF caused inhibition in cells from 2 of 3 cows only. Cells from the two cows that were inhibited by GM-CSF had higher basal secretion of prostaglandins than cells from the cow that were not inhibited (PGE$_2$: 93.3 ng/ml and 106.0 ng/ml vs. 19.1 ng/ml; PGF: 3.4 ng/ml and 2.9 ng/ml vs. 1.3 ng/ml).

**Effects of IL-2 and TNF-α.** There were no significant effects of either of these cytokines on secretion of PGE$_2$ or PGF from epithelial or stromal cells (Figure 1).

**Discussion**

These results provide evidence that cytokines can regulate prostaglandin secretion in bovine endometrial tissue and that the pattern of regulation depends upon the cytokine and cell type involved. As for many other cells (12,26,27), including human endometrial epithelial cells (10), IL-1 caused a large increase in PGE$_2$ secretion from epithelial cells. It also stimulated PGF secretion,
Prostaglandin secretion from epithelial cells and stromal cells following treatment with various cytokines. Data represent least-squares means ± pooled SEM of cultures from 2-4 cows. Note the inset graph in the panel for GM-CSF in which values for PGF secretion from stroma have been redrawn at a different scale to better display the effect of GM-CSF.

though to a much smaller degree than PGE₂. There was no effect, however, of IL-1 on secretion of prostaglandins from stromal cells. One possibility is that stromal cells do not express receptors for IL-1. Alternatively, receptors are present but the actions of IL-1 on prostaglandin secretion are abrogated in these cells. The effects of GM-CSF were also dependent on cell type, with GM-CSF inhibiting secretion of prostaglandins from stromal cells while having no effect on prostaglandin secretion from epithelial cells. The inhibitory effect of GM-CSF on prostaglandin secretion is in contrast to its actions on other cells including polymorphonuclear leukocytes (13) and mononuclear phagocytes (14), where it increases PGE₂ secretion. Similarly, IL-2 and TNF-α, which have been reported to increase PGE₂ secretion in other cells (11,12,13), did not alter prostaglandin secretion from epithelial or stromal cells of the bovine endometrium.

The physiological significance of the present results is unclear. As described in the introduction, uterine and embryonic prostaglandins play important roles in several aspects of reproduction in domestic ruminants and other species. It is conceivable that IL-1 and GM-CSF transmit information between stroma and epithelium. While sources of endometrial GM-CSF in the cow have not been established, possible sources of endometrial IL-1 such as endothelium (28,29), fibroblasts (30) and macrophages (2,31) are in the stroma while the responsive cell type for prostaglandin regulation is the epithelium. The conceptus is also a possible source of IL-1 (1,32).
It is now of importance to establish whether IL-1 and GM-CSF are produced by the bovine endometrium or placenta, and if so, to determine the cell types involved and the physiological basis for regulation of their secretion.

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